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Use of Trace Elements in the Larval Shell as a Marker of Bivalve Dispersal

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LONG-TERM GOAL

Our long-term goal is to understand how hydrodynamically-mediated exchange of organisms between benthic habitats affects the dynamics and genetics of their populations. To do this, we are developing a tool for tracking larval dispersal. We are looking specifically at uptake of trace elements into the larval shells of bivalves and evaluating whether this tag can be used to identify the bivalves' source habitats in coastal environments.

OBJECTIVES

The main objectives of this project are to determine whether the initial shell of a larval bivalve incorporates trace elements from its fluid environment, and whether the suite of these elements provides a unique fingerprint that can be used as a marker of the bivalve's source habitat. To do this, we are using bivalve larvae reared in the laboratory under controlled trace-element conditions and juveniles collected in sites that are likely to vary in environmental trace elements. We analyze the trace elements in the shells using the new Finnigan Inductively Coupled Plasma Mass Spectrometer (ICP/MS) in the WHOI Chemistry Department and the large-format Cameca 1270 Ion microprobe in Geology and Geophysics, in order to accomplish three specific goals:

1. Select a suite of trace elements (out of a set that are typically enriched in anthropogenically impacted marine environments) that are incorporated into the initial shell (prodissoconch) of larval bivalves in levels proportional to their concentrations in the surrounding seawater and that can be measured using the ICP/MS and the Ion Microprobe.
2. Assemble a reference library of larval trace-element fingerprints from shells collected at coastal locations that vary in their environmental trace-element load. These fingerprints will be used for subsequent comparison to field-collected larvae and prodissoconchs of juveniles in order to determine the location of their source populations
3. Quantify trace element concentrations in prodissoconchs using the Ion Microprobe

APPROACH

To examine trace-element uptake into larval bivalve shells, we use hatchery-spawned larvae of the soft-shell clam, *Mya arenaria.* Aliquots of fertilized embryos are transferred into beakers of filtered seawater, and each beaker is spiked with one of a suite of trace elements (e.g., Ag, Cd, Co, Pb). We select trace elements that have been shown to produce linear calibration curves on the ICP/MS, and are known to vary geographically in coastal sediments, and use concentrations that cover a range of realistic values reported from field investigations of seawater and sediment pore waters. The embryos are maintained at roughly 20°C until the initial larval shell is deposited (approximately 30-36 hours). Once the larval shell is deposited, the larvae are Millepore filtered, dried, cleaned of tissue, and dissolved in acid in preparation for aspiration directly into the ICP/MS. All of these procedures are conducted using clean technique in order to minimize the potential for trace-element contamination.

To determine a trace-element fingerprint for larvae spawned in specific types of habitats and individual locales, we collect juveniles from those sites and analyze their shells on the ICP/MS and ion microprobe. Once these location-specific fingerprints are established, we compare them to field-collected larvae and protoconchs of juveniles in order to establish the habitat type and/or geographic location of their spawning grounds.

WORK COMPLETED

To date, we have completed three experimental spawnings; one pilot trial using *Mercenaria mercenaria* and two controlled experiments using the target species *Mya arenaria.*

These laboratory-spawned larval specimens have been analyzed in the ICP/MS uptake of selected trace elements into the larval shells has been documented.

Larval shells from these experiments also have been analyzed for Co and Pb uptake on the Ion Microprobe.

Individual field-collected juvenile shells (from pristine and anthropogenically impacted sites) have been analyzed for Co and Pb uptake in both the ICP/MS and the Ion Microprobe.

RESULTS

We found that trace-element concentrations in the larval shells from the spiked treatments were substantially elevated over controls for some, but not all, elements. For instance, in one representative experiment, shell trace-element levels tended to be notably higher than controls for Pb and Co, but only slightly higher for Cd and Ag (Fig. 1). Larval survivorship was high and consistent across treatments and larval appearance was normal, suggesting that none of the spiked concentrations was lethal. Variation among replicates, however, was very high and none of the differences was significant (Mann-Whitney U test, $P < 0.05$). Nevertheless, we are pleased with these results because they demonstrate two important features of our experimental design - larvae do indeed incorporate the trace elements into their shells, and the ICP/MS is sufficiently sensitive to detect trace elements in the very small shells.

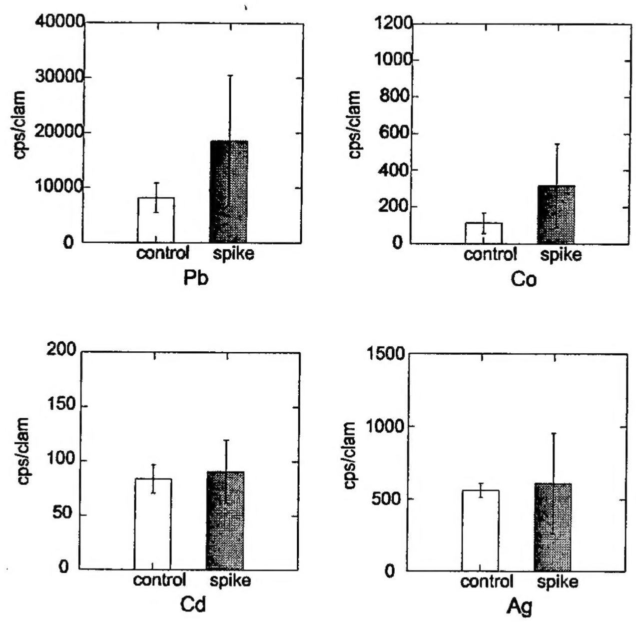

Fig. 1. ICP/MS analysis of trace elements in larval bivalve shells incubated in seawater from Vineyard Sound spiked with Pb, Co, Cd, Ag or nothing (control). Concentrations are presented as counts per second (cps); values are mean and standard error for 4 replicate beakers (except n=3 for Pb spike and n=5 for Co spike). In this trial, up to 20 shells were analyzed per beaker.

During the past year we have concentrated our efforts on analyzing larval and juvenile *M. arenaria* shells on the Ion Microprobe. In order to do this, we had to develop methods for mounting the shells in the proper orientation while minimizing loss and breakage (which was substantial, particularly among the larval shells). To date, we have conducted successful analyses targeting the prodissoconch I, comparing Pb and Co content of trace-element impacted specimens against 'clean' controls. We determined relative concentration of each specific trace element by calculating the ratio of that trace element to a calcium oxide that was close to it in molecular weight. These results indicated that Co was present at higher levels in spiked larval *Mya* shells than in the controls. We also found higher levels of Pb in juvenile clams collected from a polluted site south of Boston (e.g., Fig. 2) than in those from a relatively clean site on the north side of Cape Cod. These results are consistent with independent measures of Co and Pb in bulk-shell analyses using the ICP/MS.

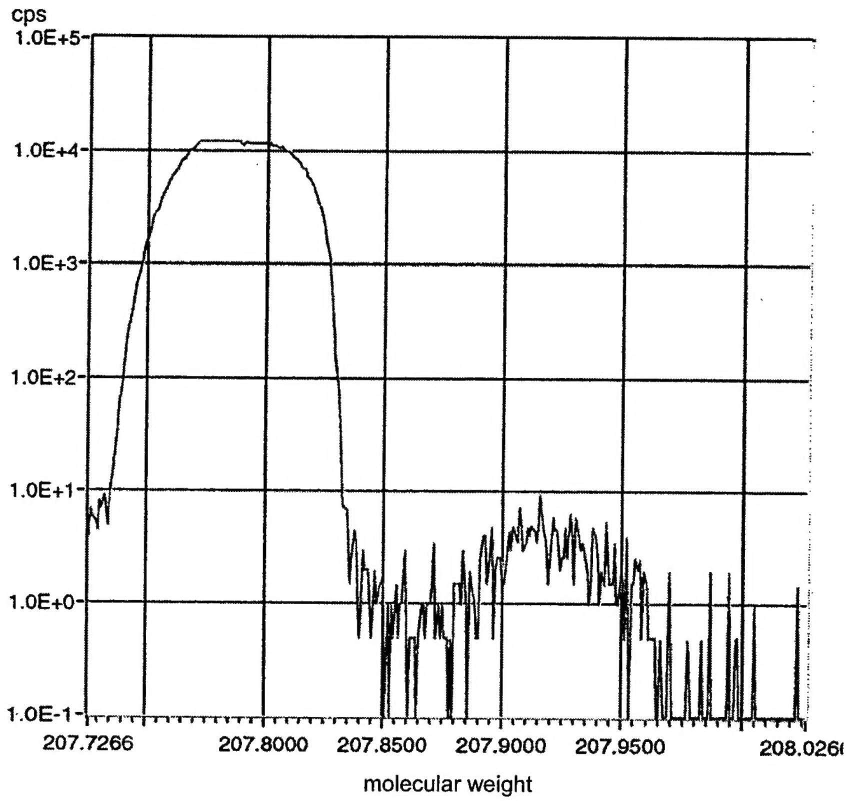

Fig. 2. Ion Microprobe scan of juvenile shell collected in polluted site south of Boston. Trace shows Pb (~207.9) and nearby calcium oxide (~207.8) for reference.

IMPACT/APPLICATION

Researchers have been searching for decades for a useful marker for larval dispersal studies, but few such markers have shown any promise in field studies. We believe we are now in a position to develop such a marker for bivalves by focusing on trace-element incorporation by the larval shell (rather than the problematic soft tissue), and by utilizing the highly sensitive analytical facilities available at WHOI. The ability to identify the source habitat of larvae will give researchers a powerful, direct tool for tracking larval dispersal in coastal waters, and for understanding the interaction of hydrodynamics and behavior in the dispersal process. It will also provide a direct measurement of the migration of individuals and gene flow among coastal bivalve populations, which can be a critical component of studies of population dynamics and population genetics. The tool has an application for assessing the impact of pollutant transports on the benthic environment in harbors; it can be used to track the export of pollutant-impacted organisms and predict the potential effects on neighboring communities.

TRANSITIONS

Once we have developed the larval marker technique, we anticipate it will be of interest to researchers conducting a wide variety of studies on hydrodynamically-mediated dispersal, population genetic exchange and population dynamics of benthic species.

RELATED PROJECTS

Our project was inspired by the successful use of this approach by fish ecologists, who use the trace-element composition of the otolith to track fish migratory paths. A similar approach is currently being used by Lisa Levin (SIO) and Claudio DiBacco (WHOI) to evaluate trace-element uptake in mussel larvae.

We are expanding the scope of this ONR-funded laboratory research to evaluate the maternal contribution to trace-element composition of the larval shell. To do this, we are repeating the spawning experiments using adults from anthropogenically-impacted habitats and comparing the shells of their larvae to those from clean habitats. Our ONR studies provided sufficient pilot data to obtain Sea Grant funding to explore this question of maternal contribution.

We are also conducting long-term studies on settlement and post-settlement processes in the soft-shell clam in a coastal harbor, funded by NOAA Sea Grant. The results of the present ONR study will allow us to incorporate the influence of larval immigration from remote habitats into our analysis of recruitment and population dynamics.

PUBLICATIONS

Mills, S., J. Blusztajn, L. Mullineaux and S. Hart, 2000. Trace-element composition in larval bivalve shells as an indicator of dispersal. Abstract volume, Larval Biology Meetings, Santa Cruz, California, June 2000

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